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double-labeled with 2-(4'-(2''-iodoacetamido)phenyl)aminonaphthalene-6-sulfonic acid (IAANS) was greater at higher temperatures, i.e. 0.11, 0.25 and 0.66 log units at 21°, 30° and 45°C respectively. In cardiac skinned fibers at higher temperatures, Ca^{2+} binding affinity (-log Ca50) was further increased for D145E (from pCa50 5.55 ± 0.01 at 15°C to 6.34 ± 0.02 at 30°C) than for WT (pCa50 5.39 ± 0.01 at 15°C to 5.96 ± 0.04 at 30°C), so that ΔpCa50 measured at different temperatures increased in a linear fashion. Maximal tension (P0) was markedly lower for D145E compared to WT at 30°C. These data indicate that D145Es instability dictates its impaired function within the physiological milieu. NIH-HL103840 (JRP)

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Remote Ischemic Conditioning Restores Contractile defect Following Ischemia by Reversing Ischemia-Induced Decreases in Phosphorylation of Sarcomeric Proteins

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Non-invasive remote ischemic conditioning, repetitive brief ischemia of the arm or leg through alternating inflation and deflation of a blood-pressure cuff, has been demonstrated to produce cardioprotection in animal models and in clinical studies. Our previous study using an *in vivo* rat ischemia model demonstrated a decrease in maximal force per cross-sectional area (Fmax) and the rate constant of force activation (k_{act}) following flash photolysis of caged Ca^{2+} (NP-EGTA), which correlated with a decrease in phosphorylation of troponin I (TnI) and myosin binding protein-C (MyBP-C), important regulators of muscle contraction and targets of the cAMP-dependent protein kinase (PKA) upon β-adrenergic receptor (β-AR) stimulation. Therefore, we hypothesized that remote ischemic conditioning would mitigate the decrease in mechanical function following acute ischemia by reversing ischemia-derived decreases in phosphorylation of sarcomeric proteins and/or of PKA subunits. To test this hypothesis, we examined Fmax, k_{act}, and phosphorylation status of MyBP-C, TnT, TnI, and PKA subunits in control, ischemic and conditioned rat myocardia. Remote ischemic conditioning was simultaneously applied to experimental rats undergoing 30-min acute myocardial ischemia with LAD ligation by executing 3 cycles of 5-min inflation and deflation of a blood-pressure cuff mounted on the left hind limb. Our data show that remote ischemic conditioning significantly blunts the reduction in Fmax (72.5 ± 4.9 vs 53.7 ± 3.4 vs 64.9 ± 2.4 mN/mm^2; control vs ischemic vs conditioned, n=9; $P < 0.05$) and in k_{act} (24.4 ± 1.9 vs 19.1 ± 1.0 vs 23.1 ± 1.1 s^{-1}; control vs ischemic vs conditioned, n=9; $P < 0.05$), which correlates with reversal of ischemia-derived decreases in phosphorylation of target proteins of PKA. These data suggest that remote conditioning merits additional study for its beneficial role in ischemia-induced myocardial dysfunction.

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Mechanistic Understanding of the Involvement of MyBP-C Slow in the Development of Distal Arthrogryposis

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Myosin Binding Protein-C (MyBP-C), expressed in striated muscles, is a thick filament associated protein that plays important roles in thick filament assembly and stabilization, and the modulation of actomyosin cross-bridges formation. Several studies have implicated MyBP-C in the development of cardiac and recently skeletal myopathies, underlining its importance in normal sarcomeric function. Mutations in the slow skeletal isoform of MyBP-C (sMyBP-C) have been causally linked to severe and lethal forms of arthrogryposis, a family of neuromuscular diseases. Two missense mutations within MYBPC1, the gene that encodes sMyBP-C, located within the NH2-terminal M-motif (W236R) and the COOH-terminal immunoglobulin (Ig) C8 domain (Y856H) have been linked to the development of distal arthrogryposis type-1 (DA-1), a skeletal muscle disorder characterized by contractures of the distal limbs. Here we explore the molecular defects that underlie the development of DA-1. using a combination of in vitro binding and motility assays, we show that the presence of the W236R mutation abolishes the ability of the NH2-terminus to modulate the formation of actomyosin cross-bridges, while the presence of the Y856H mutation inhibits Ig domains C8-C10 from interacting with native myosin filaments. Our findings therefore suggest that distinct mechanisms may underlie the pathogenesis of DA-1 myopathy depending on the location of the individual mutation within sMyBP-C. We are currently examining the effects of overexpressing mutant sMyBP-C proteins carrying either the W236R or the Y856H mutation in a sMyBP-C-null background, via in vivo gene transfer. In summary, our studies are the first to provide a mechanistic interpretation of the pathogenesis of distal arthrogryposis.

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How Muscle Exploits the Steady State to Regulate Contraction

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Activation of muscle requires myosin binding. The active state of muscle is stabilized by high affinity rigor bonds at equilibrium but, the steady-state catalytic cycle of myosin ATPase decreases the lifetime of the myosin interaction by several orders of magnitude. Low myosin affinity could be a justification for including Ca^{2+} binding states in a model of activation. Our work supports a simple model for steady state activation, namely, the regulatory protein tropomyosin (Tm) exists in just two states, ground (C) and excited (M), separated by an energy barrier which can be overcome by interaction with myosin. The elongated shape of Tm allows for multiple potential myosin binding partners (U_i). During its strong binding phase of the catalytic cycle, any one myosin (U_1) combines with C to form M by an equilibrium pathway. At time, t=0, M probability, P_M=1. Although U_1 most likely decays by a non-equilibrium rate (i.e., ATP binding), the lifetime of P_M is constant. For t>0, before M decays to C, interaction with U_i in the strong binding phase can restore P_M=1. We show that an analytical function (M function) is fully derived from the combination of equilibrium and steady state pathways and the additional opportunities (second chances) to restore P_M=1 depend on the rate at which U_i transitions into the strong binding phase. When Ca^{2+} is introduced as a mass action regulator of the U or C supply, the M function takes the general form of the Hill equation, which is widely employed for fitting the steady state response of muscle to Ca^{2+}. We show by simulations that stochastic events predicted by a second chance mechanism are consistent with solutions to the M function. Our results justify kinetic experiments that focus specifically on M.

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Troponin I Serine 150 Phosphorylation Inhibits pH-Induced Troponin Calcium Desensitization

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A hallmark of cardiac ischemia is decreased intracellular pH which can affect a number of cellular processes. Such an acidic environment alters cardiac troponin (Tn) myofilament regulation to decrease the Ca^{2+} sensitivity of force production. Tn also undergoes cardiac ischemic-induced AMPK troponin I (TnI) Ser-150 phosphorylation. We recently characterized the effect of TnI Ser-150 phosphorylation demonstrating it to increase myofilament Ca^{2+} sensitivity and blunt TnI PKA phosphorylation-induced desensitization. The effect of an acidic environment on Tn myofilament regulation in the presence of the ischemic-induced TnI Ser-150 phosphorylation is unknown. In this study we investigate the effect of pH on myofilament regulation in the presence of TnI Ser-150 phosphorylation. Myofilament regulation was determined by measuring troponin C Ca^{2+} binding properties at normal and acidic pH. Results demonstrate acidic pH decreases steady-state Ca^{2+} binding to troponin C in thin filaments reconstituted with either WT or Ser-150 pseudo-phosphorylated TnI (Tn S150D) such that Tn S150D Ca^{2+} sensitivity at pH 6.5 is similar to WT at pH 7. Furthermore, while Tn S150D slowed Ca^{2+} dissociation compared to Tn WT at pH 7.0, decreasing the pH to 6.5 did not further alter isolated Tn S150D Ca^{2+} dissociation indicating a lack of pH to modulate Tn Ca^{2+} dissociation kinetics. Thin filament dissociation kinetics are ongoing. We conclude that TnI Ser-150 phosphorylation imparts resistance to acidic pH induced myofilament Ca^{2+} desensitization similar to that present during ischemia without alteration of Tn Ca^{2+} dissociation. Future investigations are aimed at examining the effect of TnI Ser-150 phosphorylation in the presence of TnI Ser-23/24 phosphorylation at low pH.

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Phosphate Enhances Regulated Thin Filament Velocity at Acidic pH in the Motility Assay

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Elevated levels of inorganic phosphate (P_i) and hydrogen ions (H^+) are putative agents of muscle fatigue. However, the molecular mechanisms underlying their inhibitory action on the contractile proteins remains unclear. To gain mechanistic insight into the effects of these ions on the myofilament function, we studied their impact on the velocity-pCa relationship in an *in vitro* motility assay. In this assay, actin filaments reconstituted with fast skeletal troponin (Tn) and tropomyosin (Tm) moved on a coverslip surface coated with skeletal myosin. At saturating Ca^{2+} (pCa5), decreasing the pH from 7.4 to 6.8 and